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Sent: Friday, October 03, 2003 5:24 PM  
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AN 1999261970 MEDLINE  
DN 99261970 PubMed ID: 10330243

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SO AMERICAN JOURNAL OF PHYSIOLOGY, (1999 May) 276 (5 Pt 2) H1591-8.  
Journal code: 0370511. ISSN: 0002-9513.

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# The effects of mannitol, albumin, and cardioplegia enhancers on 24-h rat heart preservation

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**Dunphy, Gail, Helen Wilkinson Richter, Masoud Azodi, John Weigand, Fereydoon Sadri, Frank Sellke, and Daniel Ely.** The effects of mannitol, albumin, and cardioplegia enhancers on 24-h rat heart preservation. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H1591–H1598, 1999.—During 24 h in vitro heart preservation and reperfusion, tissue damage occurs that seriously reduces cardiac function. Prevention of free radical production during preservation and reperfusion of ischemic tissue using free radical scavengers is of primary importance in maintaining optimal heart function in long-term preservation protocols. We examined whether mannitol (68 mM) and albumin (1.4  $\mu$ M) in combination with other cardioplegia enhancers decreased free radical formation and edema and increased cardiac function during 24-h cold (5°C) heart preservation and warm (37°C) reperfusion in the Langendorff-isolated rat heart. The performance of mannitol-treated hearts was significantly decreased compared with that of hearts without mannitol treatment after 24 h of preservation with regard to recovery of diastolic pressure, contractility (+dP/dt), relaxation (–dP/dt), myocardial creatine kinase release, coronary flow, and lipid peroxidation. Albumin-treated hearts demonstrated higher cardiac function (contractility and coronary flow especially) than hearts not treated with albumin or hearts treated with mannitol, and this appears to be due to the positive effects of increased cellular metabolism and the enhancement of membrane stability.

organ preservation; free radicals; reperfusion injury; scavengers

IN VITRO HEART PRESERVATION for 24 h produces unique problems involving altered energy metabolism, cell organelle and membrane damage, left ventricular dysfunction, and reperfusion injury. Recent studies have evaluated various cardioplegia solutions (10, 34) and specific enhancers (2) that are intended to protect the heart from preservation-induced injury. Several studies have also described prevention of reperfusion damage through the use of chelators (6) and enzymes (11, 32) selected to reduce the concentration of free radicals. Additional problems are encountered after 24 h of heart preservation, such as the short biological half-life of many enzymes that scavenge free radicals, antigenicity of enzymes, and pH and dosage effects that can accumulate during that period. We found that five additives to a standard cardioplegia solution (insulin, ATP, corticosterone, pyruvate, and the iron chelator deferoxamine) maintained 24-h rat heart preservation at ~90% of function (8).

Several studies have suggested that albumin and mannitol may reduce ischemic and reperfusion injury. The proposed mechanisms of action of mannitol are the prevention of edema by hyperosmotic action (19, 26, 35) and the reduction of lipid peroxidation by scavenging hydroxyl radicals (29, 33). The beneficial effects of mannitol appear to be dose dependent, and the optimal mannitol dosage for reducing ischemic-induced ventricular fibrillation was 50 mM (35). Gronow et al. (12) showed that a dose of 1–10 mM mannitol minimized edema and reduced malondialdehyde (MDA) formation (lipid peroxidation index) in the postischemic kidney model; however, unfavorable cell membrane effects were observed at higher doses (>60 mM). Magovern et al. (19) showed that mannitol produced greater coronary flow and less edema after 60 min of hypothermia (27°C) and cardioplegic arrest in rabbit hearts compared with those hearts treated with isosmolar or hyperosmolar glucose. This study and others (22) suggest that mannitol exerts its beneficial effects during early reperfusion by reducing edema and possibly free radical formation.

The proposed mechanisms of action for the beneficial effect of albumin in cardioplegia solutions are the maintenance of a high osmotic pressure and the reduction of free radical formation (27). However, the effectiveness of mannitol and albumin during longer periods of preservation (24 h) has not been ascertained. Therefore, the objective of the following study was to determine the effectiveness of mannitol and albumin with standard and enhanced cardioplegia during 24-h rat heart preservation.

## MATERIALS AND METHODS

**Experimental groups.** Seven groups of rats ( $n = 6$  rats/group) were tested for left ventricular function before and after a 24-h heart preservation routine. Biochemical indicators of tissue damage, release of creatine kinase, and MDA content of tissue were evaluated at the end of the 24-h period. All cardioplegia solutions contained the Krebs-Henseleit buffer (K) which was composed of (in mM) 119 NaCl, 15 KCl, 0.8  $\text{CaCl}_2$ , 5.2  $\text{MgCl}_2$ , 25  $\text{NaHCO}_3$ , 1.2  $\text{KH}_2\text{PO}_4$ , and 11 glucose. Some solutions contained the following enhancers, which we have previously shown to be successful in 24-h preservation: 0.12 mM corticosterone, 0.14 mM pyruvic acid, 10.4  $\mu$ M ATP, and 24 U/l insulin. We originally used 71 enhancers in the cardioplegia to benefit heart function, and from those we were able to narrow down to five the powerful additives that were necessary (9). Some solutions contained albumin (1.4  $\mu$ M), some contained mannitol (68 mM), and some contained neither of these two additives (Table 1). The seven groups

Table 1. Composition of 24-h cardioplegia solutions

Group	Enhancers	Albumin	Mannitol
K	-	-	-
KA	-	+	-
KM	-	-	+
E	+	-	-
EA	+	+	-
EM	+	-	+
EAM	+	+	+

+, With; -, without; K, Krebs-Henseleit buffer; KA, Krebs-Henseleit buffer + albumin; KM, Krebs-Henseleit buffer + mannitol; E, enhancers; EA, enhancers + albumin; EM, enhancers + mannitol; EAM, enhancers + albumin + mannitol.

consisted of two subgroups: 1) Krebs-Henseleit buffer (K), Krebs-Henseleit buffer and albumin (KA), and Krebs-Henseleit buffer and mannitol (KM); and 2) enhancers (E), enhancers and albumin (EA), enhancers and mannitol (EM), and enhancers, albumin, and mannitol (EAM). The K-group solutions do not contain the enhancers, and the E-group solutions do contain the enhancers. In RESULTS, group K and group E are used as controls for assessing the effects of albumin and mannitol on 24-h cardioplegia. In addition, we assess the effect of the enhancers by comparing group E (enhancers) with group K (no enhancers).

**Perfusion and storage of hearts.** Hearts from spontaneously hypertensive rats (SHR;  $n = 43$ , wt 250–350 g) were isolated and perfused using the Langendorff technique (25). SHR rats were used because our laboratory has studied hypertension for over 20 years in these animals and we have a large database of physiological phenotypes for comparison. Rats were injected with heparin (500 U) 5 min before anesthesia (50 mg/kg ip, Brevital, Eli Lilly, Indianapolis, IN). Hearts were removed and rinsed in an ice-cold K solution (24) containing 119 mM NaCl, 4.7 mM KCl, 2.5 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgSO}_4$ , 25 mM  $\text{NaHCO}_3$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 14 mM glucose, and 1.4  $\mu\text{M}$  albumin. Atria were trimmed, and the hearts were attached by an aortic cannula to a hydrostatic perfusion apparatus. Hearts were retrograde-perfused at 80 Torr, 37°C, with the above K solution, which was bubbled with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . A nonelastic water-filled balloon was secured in the left ventricle, and the volume was adjusted via a syringe to achieve a zero diastolic pressure as measured with a pressure transducer (Statham model P23 Db) and physiograph (4-channel recorder, Gould, Cleveland, OH). The hearts were paced at 240 beats/min using bipolar pacing (Grass Instruments, Quincy, MA). Left ventricular systolic and diastolic pressures were recorded at five balloon volumes (50–300  $\mu\text{l}$ ) yielding positive and negative first derivatives of left ventricular pressure with respect to time ( $\pm dP/dt$ ). Coronary perfusion flow was determined at the midpoint balloon volume (150  $\mu\text{l}$ ) by collection of the coronary effluent for 1 min.

After these initial pressure-volume curves were recorded, the cannulated hearts were removed from the hydrostatic apparatus and connected to a pulsatile roller pump (Minipuls 2 pump, Gilson, Middleton, WI). The hearts were perfused at a flow rate of 2 ml/min through Tygon tubing (R3603) using one of the seven cardioplegic solutions (Table 1) saturated with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . Disposable 5- $\mu\text{m}$  filters (Gilson) were placed in the perfusion line immediately before entry into the aorta. Each heart was placed in a plastic bag (Saran) that was vented at the top to ensure moistness. The entire apparatus was transferred to a cold chamber (5°C) for 24 h with continuous recirculated perfusion. The pH of the perfusate

was continuously monitored and remained at 7.4 due to the bubbling of the 95%  $\text{O}_2$ -5%  $\text{CO}_2$  mixture.

After 24 h of cold storage, the heart was removed from the cold chamber, reattached to the hydrostatic apparatus, and reperfused with warm (37°C) K plus albumin (described above) to which mannitol (68 mM) was added. The mannitol was added to make the solution hyperosmolar (330 mosM) to reduce edema (21). Pressure-volume curves and short-term coronary flow were recorded again, and reperfusion fluid was collected for creatine kinase analysis. Percentage measurements for the recovery period were made at a midpoint balloon volume of 150  $\mu\text{l}$ . The data were not corrected for the difference in the final coronary flow rates because, during the majority (99%) of the preservation period, flow was held constant at 2 ml/min. During the preservation period, the hearts were quiescent, and on reperfusion any arrhythmias stopped when 37°C was reached. Occasionally a higher voltage was used to maintain heart rate until 37°C was reached, and then the voltage was turned down to normal (4 V). Hearts were weighed before and after preservation to determine water accumulation. After the final tests, the hearts were frozen (-70°C) and later used for MDA assays.

Reperfusion fluids were analyzed for creatine kinase using the Sigma Diagnostics method (Sigma Diagnostics, St. Louis, MO, 20) that was optimized by Szasz (28). Samples were either analyzed immediately for creatine kinase or refrigerated (5°C) for no longer than 1 wk before analysis. Our data suggest that for prolonged storage the addition of 2.5 g/dl albumin is required to stabilize the enzyme (7).

Whole heart MDA was measured as an indicator of lipid peroxidation by the thiobarbituric acid (TBA) method first developed by Kohn and Liversedge (17) for brain tissue and later modified for other biological tissues (4). Kosugi and Kikugawa (18) analyzed the potential MDA reactive substances in peroxidized lipids and showed that the TBA method measures lipid oxidation. There has been some controversy as to the sensitivity of MDA as a marker for lipid peroxidation at low free radical levels (3); however, we feel it provides an adequate marker in 24-h studies with elevated lipid peroxidation. In brief, the heart tissue was prepared by first removing the aorta and cutting the heart into pieces. The pieces were then placed in a large mortar, covered with liquid nitrogen, and ground into a fine powder. The powdered tissue was mixed with water (ratio of tissue to water 1:9 by mass) to lyse the cells. The supernatant was added to a TBA-TCA solution and heated for 1 h at 95°C. The supernatant was removed from the flocculent precipitate, and the absorbance was determined at 535 nm against a sample blank containing all of the reaction constituents minus the TBA (4). The MDA concentrations were calculated from a standard curve prepared with 1,1,3,3-tetramethoxypropane (Sigma).

Results were analyzed for significance by one-way ANOVA, followed by the Student-Newman-Keuls method for pairwise multiple comparisons, and are expressed as means  $\pm$  SE. All experiments were performed according to the guidelines of "The Principles of Laboratory Animal Care" (NIH), and procedures were approved by the University of Akron Animal Use and Care Committee.

## RESULTS

The results of the cardioplegia comparisons are summarized in Table 2. The percent recovery of contractility ( $+dP/dt$ ) and relaxation ( $-dP/dt$ ) after 24 h of preservation for each group is shown in Figs. 1 and 2, respectively. The results for both contractility and relaxation were similar. The addition of albumin (KA)

Table 2. Summary of results

Parameter	KA vs. K	KM vs. K	EA vs. E	EM vs. E	EAM vs. E	E vs. K
% (+dP/dt) recovery	=	=	≥	<	=	=
% (-dP/dt) recovery	=	=	>	<	=	=
%Coronary flow recovery	=	=	>	=	=	=
Diastolic pressure increase, Torr	=	≥	≤	=	=	>
%Systolic pressure recovery	=	=	<	<	≤	>
%Mass gain	=	=	=	=	=	=
Creatine kinase, U/g	=	=	<	=	=	=
Malondialdehyde, nmol/g	≤	=	=	>	≥	<

+dP/dt, left ventricular contractility; -dP/dt, left ventricular relaxation. Symbols (=, >, ≥, <, ≤) compare 1st vs. 2nd group value in each column: =, no significant difference ( $P > 0.05$ ); > and ≥, significant increase ( $P < 0.05$  and  $P < 0.01$ , respectively); < and ≤, significant decrease ( $P < 0.05$  and  $P < 0.01$ , respectively).

or mannitol (KM) to the Krebs buffer had no significant effect in the Krebs groups (K). The addition of albumin to the E group (EA) produced a large increase in the percent recovery of contractility ( $P < 0.05$ ). The enhanced mannitol-containing solution (EM) showed a decrease in the percent recovery ( $P < 0.05$ ). When both albumin and mannitol were added to the enhancers (EAM), the positive albumin effect and the negative mannitol effect canceled each other out, and group EAM was not significantly different from group E. Group E and group K showed about the same contractility.

The percent recovery of coronary flow is shown in Fig. 3. As previously described, the addition of albumin (KA) or mannitol (KM) to the K solution had no

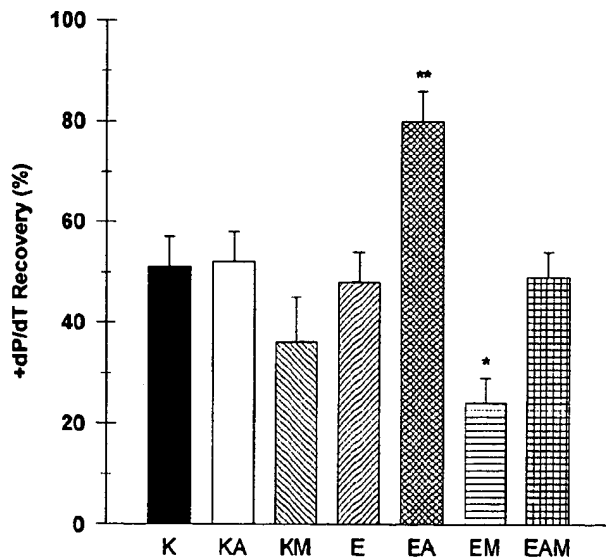


Fig. 1. Left ventricular contractility (+dP/dt) recovery (%) in each treatment group after 24-h heart preservation. K, Krebs-Henseleit solution; KA, Krebs + albumin; KM, Krebs + mannitol; E, enhancers; EA, enhancers + albumin; EM, enhancers + mannitol; EAM, enhancers + albumin + mannitol. Values are means  $\pm$  SE. \*  $P < 0.05$ , \*\*  $P < 0.01$  compared with group E.

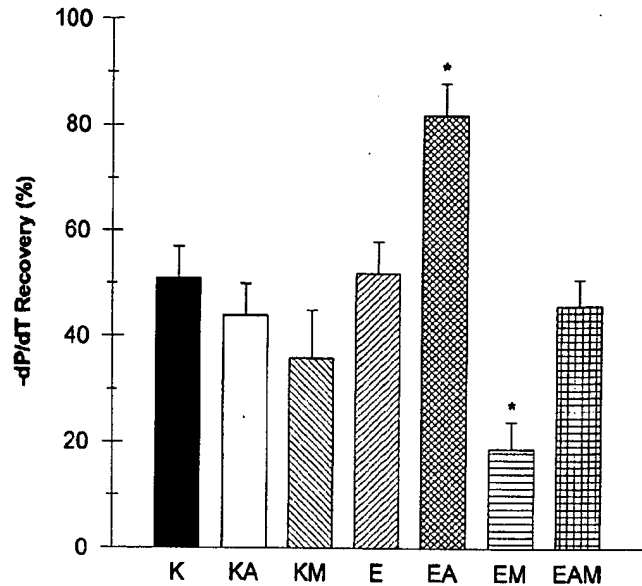


Fig. 2. Left ventricular relaxation (-dP/dt) recovery (%) in each treatment group after 24-h heart preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  compared with group E.

significant effect in the K groups. The addition of albumin in the E group (EA) produced a large (49%) increase in the percent recovery of coronary flow ( $P < 0.05$ ). The addition of mannitol (EM) gave the same result as the control (E), as did the addition of both albumin and mannitol. The percent recovery of group E was similar to that of group K (51% and 61%, respectively).

The change in diastolic pressure (Torr) is shown in Fig. 4. In all groups the diastolic pressure increased after 24 h. In the K groups, the addition of albumin (KA) had no effect, but mannitol (KM) produced a very

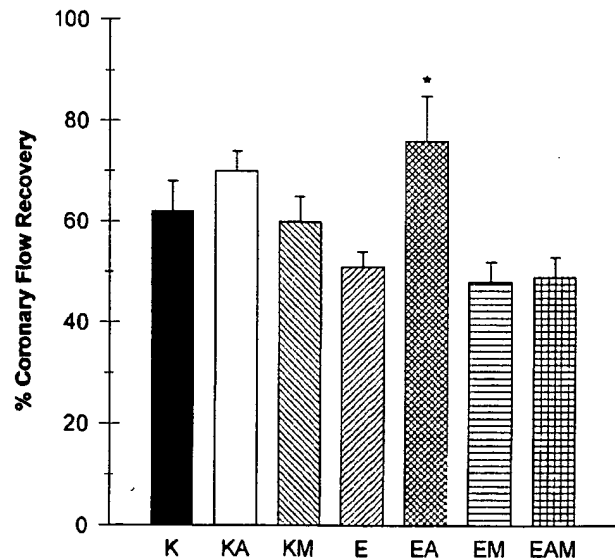


Fig. 3. Percent coronary flow recovery in each treatment group after 24-h heart preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  compared with group E.

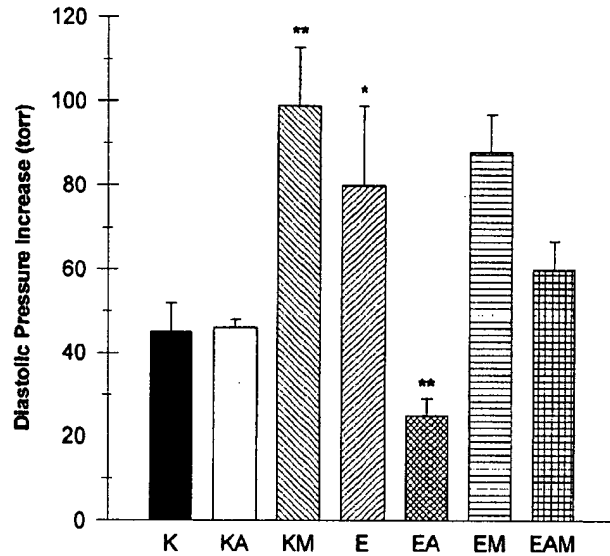


Fig. 4. Left ventricular diastolic pressure (actual increase in pressure compared with initial *time 0* value) in each treatment group after 24-h heart preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  for E vs. K; \*\*  $P < 0.01$  for EA vs. E and for KM vs. K.

large increase (120%) in diastolic pressure ( $P < 0.01$ ). In the E groups, the addition of albumin (EA) greatly reduced (69%) the postcardioplegia diastolic pressure increase ( $P < 0.01$ ). The addition of mannitol (EM) had no effect, although the addition of both albumin and mannitol (EAM) gave a small nonsignificant decrease (24%), i.e., the mannitol counteracted some of the positive effect of the albumin in group EAM. The addition of enhancers to the K solution (group E vs. group K) caused a large increase (78%) in the postcardioplegia diastolic pressure ( $P < 0.05$ ).

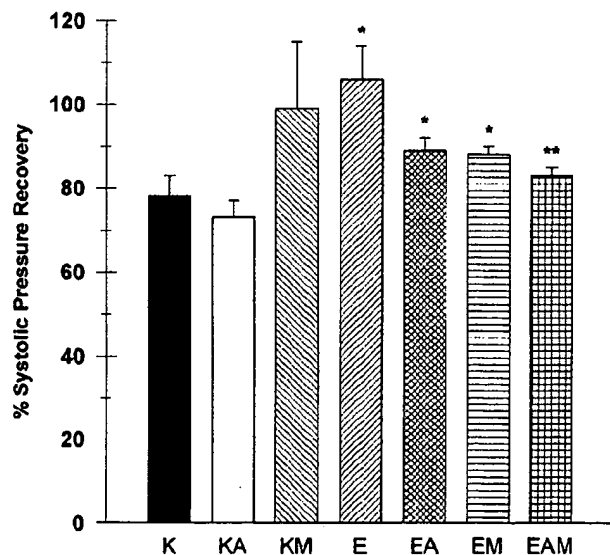


Fig. 5. Left ventricular systolic pressure recovery (percentage of initial *time 0* value) for each treatment group after 24-h heart preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  for E vs. K, for EA vs. E, and for EM vs. E; \*\*  $P < 0.01$  for EAM vs. E.

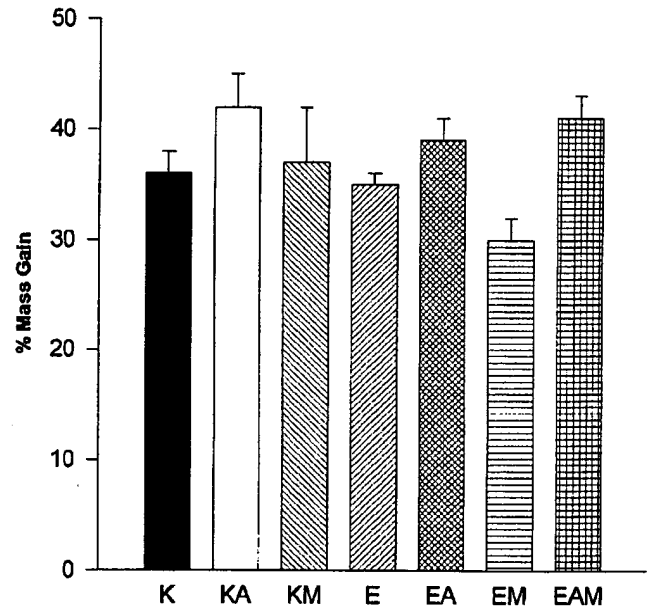


Fig. 6. Percent mass gain recovery of initial *time 0* value compared with 24-h preservation value. Values are means  $\pm$  SE; no significant differences.

The percent recovery of systolic pressure is shown in Fig. 5. There were no significant differences among the K groups. Groups EA and EM showed a significant reduction (15%) compared with group E ( $P < 0.05$ ), and group EAM showed a larger decrease in function (22%,  $P < 0.01$ ). Group E had a 33% increase in systolic function compared with group K ( $P < 0.05$ ). There was no significant difference among any of the groups in percent mass gain (Fig. 6).

The postpreservation efflux of creatine kinase is shown in Fig. 7. In the K groups, the addition of

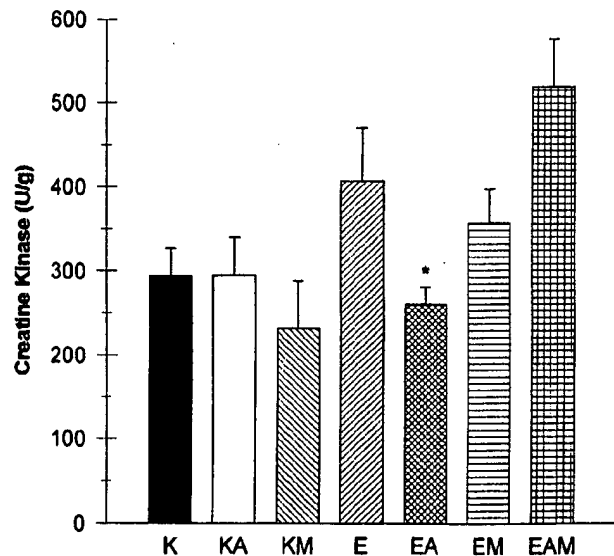


Fig. 7. Creatine kinase levels (U/g heart) released to the perfusate in each treatment group on reperfusion after 24-h preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  for EA vs. E.

albumin (KA) or (KM) mannitol had no significant effect. In the E groups, albumin (EA) produced a substantially reduced (37%,  $P < 0.05$ ) creatine kinase value, whereas mannitol (EM) had no effect; the combination of albumin and mannitol (EAM) had no effect. The addition of enhancers to *group K* caused a nonsignificant increase in the creatine kinase efflux.

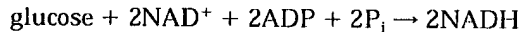
The production of MDA in heart tissue is shown in Fig. 8. In the K groups, the addition of albumin (KA) caused a large decrease (39%,  $P < 0.01$ ) in MDA, but there was no significant difference with the addition of mannitol (KM). In contrast, in the E groups, the addition of mannitol (EM) produced an increase in MDA (60%,  $P < 0.05$ ), and the addition of both albumin and mannitol (EAM) produced an even larger increase in MDA (116%,  $P < 0.01$ ). The addition of enhancers to the K solution reduced MDA production (31%,  $P < 0.05$ ).

## DISCUSSION

*The effects of albumin in 24-h preservation.* In summary, albumin enhanced heart function through its role as an antioxidant and increased cell metabolism protection after increased energy supply provided by the enhancers. In the absence of the enhancers, albumin in K (*group KA*) had no effect on any of the physiological parameters with the exception of a reduction in MDA. In stark contrast, albumin produced marked improvement in four of the parameters in the presence of the enhancers (*group EA*). The improvements of these parameters are not due to the enhancers alone, because their addition to K produced no or only slightly negative changes in the physiological parameters (*group E* vs. *group K*). Clearly, there is a synergistic effect

between albumin and one or more of the enhancers: insulin, pyruvate, ATP, and corticosterone.

What are the expected actions of the enhancers on the heart? Insulin stimulates muscle cells to take up glucose: the administration of insulin can increase the rate of passive-mediated glucose transport into the cells by an order of magnitude. This increase in cellular glucose will stimulate metabolism via the glycolytic pathway, where the overall reaction is (30)



Under anaerobic conditions,  $\text{NAD}^+$  is regenerated when NADH reduces pyruvate to lactate, although under aerobic conditions mitochondrial oxidation of NADH yields three ATPs. During preservation, the cellular oxygen levels are below normal so that substantial pyruvate will be consumed in reducing  $\text{NAD}^+$ , although the production of ATP will be reduced. The addition of pyruvate and ATP to the perfusate can compensate for the losses of pyruvate and ATP required for cell function, provided they can cross the cell membrane. A deficit of glucocorticoids, such as corticosterone (e.g., in disease states such as Addison's disease), is characterized by hypoglycemia, muscle weakness,  $\text{Na}^+$  loss,  $\text{K}^+$  retention, and impaired cardiac function; the addition of corticosterone may compensate for membrane stabilization and energy loss during cardioplegia. In summary, the expected actions of the enhancers are stimulation of metabolism in the heart muscle cells by insulin, pyruvate, and ATP and regulation of cell functions by corticosterone. As noted above, the observed actions when just the enhancers were added to the K solution were essentially nil or slightly negative with regard to the physiological function parameters.

What are the normal functions of albumin and what pathways are there for synergism between albumin and one or more of the enhancers? Albumin is a water-soluble protein that makes up about one-half of the blood-serum protein. In addition to regulation of osmotic pressure, albumin plays an important membrane transport function (30). Steroids such as corticosterone can be transported in the blood and be made accessible to cells in complex with albumin. Albumin binds free fatty acids in the blood; when the fatty acid concentration is too large, they form micelles that act as detergents to disrupt protein and membrane structure (30). Human albumin has specific sites for binding of copper ions. Albumin can scavenge hypochlorous acid and thereby prevent damage to  $\alpha_1$ -protease (15). Albumin can inhibit peroxidation (14), exhibit antioxidant capacity (13), decrease lipoxygenase activity (31), and act as a coenzyme for tissue-repair enzymes. In general, proteins such as albumin probably play a protective role in vivo by acting as "sacrificial antioxidants" (15).

As noted above, the addition of albumin alone to the K solution had no effect on the physiological parameters, but the production of MDA was reduced. This is consistent with albumin simply acting as a sacrificial

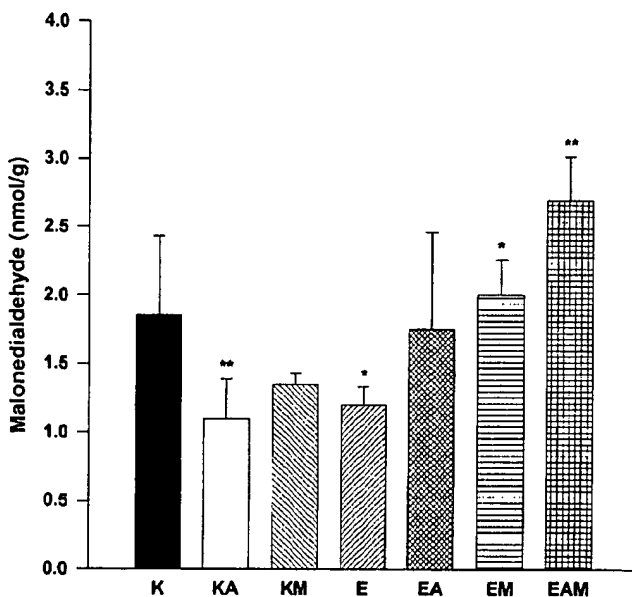


Fig. 8. Myocardial malondialdehyde values in each treatment group after 24-h heart preservation. Values are means  $\pm$  SE. \*  $P < 0.05$  for E vs. K and for EM vs. E; \*\*  $P < 0.05$  for KA vs. K and for EAM vs. E.

antioxidant, although the reduction in peroxidation was not sufficient to be reflected in the physiological parameters measured.

For *groups E* and *EA*, the expected actions of the enhancers and of albumin and their observed synergism suggest the following conclusions. The enhancers, particularly insulin, pyruvate, and ATP, stimulate metabolism in the heart muscle cells. This is both good and bad. The stimulation of metabolism should increase the viability of the cells; however, when metabolism was increased, the oxygen deficit in the already-ischemic cells was increased. Thus in *group E* hearts the positive and negative effects essentially cancel each other out with regard to the physiological parameters, except in the percentage of coronary flow recovery and diastolic pressure increase, where small negative effects were observed (*group E* vs. *group K*). A decrease in MDA appeared, just as for *group KA*, which can be attributed in *group E* to insulin acting as a sacrificial oxidant. When albumin was introduced, the negative effects were offset, whereas the positive effects were unmasked. Why is this so? One potential mechanism is that the increased rate of metabolism stimulated by the enhancers produced increased free radical production with concomitant increases in lipid peroxidation pathways: thus the very slight negative effect of the enhancers on the physiological parameters. The addition of albumin gave protection from the negative effects of lipid peroxidation, expected from the above discussion, thus allowing the positive effects of increased cellular metabolism to emerge. Albumin acts as a competing sacrificial oxidant and thereby protects the insulin concentration, thus promoting an increase in metabolism above that seen in *group E*. This increase in metabolic rate for *group EA* results in an increase in the number of viable cells, reflected in the physiological parameters by a concomitant increase in the absolute amount of peroxidation, which is reflected in the slightly increased MDA. In addition to promoting an increased metabolic rate, albumin should enhance the positive effects of corticosterone by promoting its transmembrane mobility. The seemingly contradictory result of slightly (nonsignificantly) increased MDA production and decreased creatine kinase release might be explained in that MDA reflects the cumulative 24-h biochemical peroxidation, creatine kinase reflects the structural cell damage on reperfusion, and severe cell damage has not yet occurred.

*The effects of mannitol on physiological parameters in 24-h preservation.* In summary, mannitol decreased heart performance when interacting with the enhancers most likely by reducing energy substrates to the cells and potentially causing cell damage in the long term. In the absence of the enhancers, mannitol in *K* (*group KM*) had no effect on any of the physiological parameters except for a large increase in diastolic pressure indicative of increased heart stiffness, which was probably due to injury rather than edema because there were no significant differences between groups in water gain. As with albumin (*group KA*), mannitol produced a decrease in MDA. In the presence of the

enhancers, mannitol greatly reduced the contractility and relaxation, and significantly reduced systolic pressure recovery (*group EM*). These responses were not due to the enhancers alone because they produced little change in the physiological parameters (*E* vs. *K*). Clearly, as with albumin, there was a synergism between mannitol and one or more of the enhancers, only it was a negative synergism with mannitol.

Many compounds structurally similar to D-glucose, including D-mannitol, inhibit glucose transport across the cell membrane. The addition of mannitol to the perfusate would be expected to introduce competitive inhibition of glucose uptake into the heart muscle cells, which may not be so important in the short term, but in long-term perfusions it could lead to cell exhaustion. Many studies have shown enhanced cardiac recovery when mannitol was administered during short-term ischemia-reperfusion. The positive effects observed in the various systems included reduced MDA production (5, 19), arterial vasodilation (23), prevention of cell swelling (22, 26, 29), improved energy metabolism (12), and decreased reperfusion-induced arrhythmias (10, 35). At higher mannitol concentrations (>50 mM) and longer times, cell dehydration, membrane damage, and increased capillary permeability have been reported (1).

Some of the long-term effects of mannitol can be understood on the basis of its metabolic effects vis-à-vis inhibition of glucose uptake by the cells. In *group KM*, the increase in stiffness indicated by the large diastolic pressure increase can be attributed to damage resulting from "starvation" of the muscle cells over a 24-h period; the reduction in MDA observed arises either from the reduced metabolic rate or from sacrificial antioxidant behavior by the mannitol.

What about the negative synergism between mannitol and the enhancers? This can be observed by examining the effect of the enhancers of *group EM* with respect to *group KM*. With the addition of the enhancers, mannitol had a much more negative effect on the heart. The rate of glucose uptake by the heart muscle cell should be much larger in groups containing the enhancers because of the insulin; however, when mannitol is present, it competitively inhibits the glucose uptake. For the *group EM*, this means increased mannitol concentrations inside the cell, as well as perhaps decreased glucose. But clearly, extra tissue damage occurs with enhancers and mannitol, and so it seems reasonable to assume that the damage arises from the mannitol inside the cell, which is stimulated by the insulin. Interestingly, the percent mass gain for the *group EM* was the smallest of all the groups; however, the difference was not significant. Mannitol has been used as an agent to prevent edema in perfused organs, but does the mannitol cause dehydration of some cells? As the mannitol accumulates in the cell it may suppress any further influx of glucose and lead to cell starvation. Blocking of glucose uptake by mannitol may not matter for short time periods, and its control of osmotic pressure is beneficial, but for 24-h preservation mannitol appears harmful.

In conclusion, we have shown that mannitol (68 mM) does not offer added protection to cardioplegia during 24 h of cold storage in rat heart. In contrast, albumin (1.4  $\mu$ M) improved heart preservation and in combination with the enhancers (insulin, ATP, corticosterone, and pyruvic acid) offers the best combination of additives of the solutions tested. Our cardioplegia with enhancers and albumin preserved heart function equal to or better than that of the CP-11EB solution and intermittent perfusion intervals in cold rat heart (36); however, an iron chelator added to the cardioplegia preserves heart function even more (8). Hisatomi et al. (16) also found that albumin improved 6-h cold rat heart preservation at very high concentrations (2% and 5% did, but 7% did not). This beneficial effect may be due to synergism between the enhancers and albumin influencing myocardial glucose metabolism.

The authors thank Fieke Bryson for animal maintenance, Dean Petrinc for technical assistance, Dr. Michael Dunphy for editing, and Linda Bordenkircher and Sarah Francis for typing the manuscript.

This project was made possible by grants from the State of Ohio Academic Challenge and Research Challenge Programs and The University of Akron and by National Heart, Lung, and Blood Institute Grant R15-HL-41779-01A2 (to H. Richter).

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Received 30 July 1998; accepted in final form 13 January 1999.

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